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# Chromosomal Aberrations in a Consecutive Series of Childhood Rhabdomyosarcoma

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***Background and Procedure.*** During a 13-year period, 22 children were treated for rhabdomyosarcoma (RMS). In 18 of these patients chromosome analysis was attempted on material from tumor biopsies, fine needle aspiration biopsies and/or bone marrow samples.
***Results.*** Clonal chromosome aberrations were detected in 14 cases; 7 of 9 embryonal RMS, 6 of 8 alveolar RMS and in the single case of pleomorphic RMS. Cytogenetic failures were more frequent in fine needle aspiration biopsies than in tumor biopsies. The characteristic t(2;13) translocation was seen in 2 alveolar RMS but not in any of the other subtypes. In 3 of the embryonal RMS hyperdiploid or hypertetraploid karyotypes with few or no structural rearrangements were seen. In all 3 cases the clinical course was relatively benign, suggesting that certain karyotypic patterns in RMS may be of prognostic significance.
***Conclusions.*** Our results add to the evidence that cytogenetic analysis should be an integral part of the diagnostic examinations of children with RMS. Med. Pediatr. Oncol. 30:156–159, 1998. 

**Key words:** cytogenetics; histopathology; rhabdomyosarcoma; clinical course

## INTRODUCTION

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood and accounts for 4–8% of all pediatric malignancies [1]. RMS are histologically classified as embryonal, alveolar, or pleomorphic tumors, with considerable overlap among the entities [2]. By supplementing the histologic diagnosis with information of the chromosomal and molecular genetic aberration patterns it may be possible to obtain a more precise pathogenetic classification. Furthermore, consistent chromosomal rearrangements in RMS cells could be of prognostic importance, as has been demonstrated for other pediatric malignancies such as acute leukemias and neuroblastoma [3].

The most frequent structural chromosome aberration in RMS is the translocation t(2;13)(q35;q14), which was first described in 1982 [4]. This translocation or variants thereof, notably the t(1;13)(p36;q14), are particularly common in the alveolar subtype of RMS, but has also been detected in a few embryonal and mixed alveolar/embryonal cases [5]. At the molecular level both translocations result in the fusion of two transcription factor encoding genes; a fork head domain gene (*FKHR*) on chromosome 13 fuses with either the *PAX3* gene on chromosome 2 or the *PAX7* gene on chromosome 1 [6,7]. As a result, a novel transcription factor is produced. Sometimes the t(2;13) or the t(1;13) may be found as the sole cytogenetic anomaly, but mostly they are accompanied by other, presumably secondary, structural, and numerical alterations.

Whether the abscence or presence of primary translocations or the extent of secondary changes in any way influences the biological aggressiveness of the tumors and hence the clinical outcome remains unknown.

The present study comprised 14 short-term cultured RMS with clonal chromosome aberrations. Apart from extending the cytogenetic data base on this tumor type, the aim was to search for possible correlations between chromosomal pattern and histopathologic features or clinical course.

## PATIENTS AND METHODS

During the period 1984–1996, 22 children were treated for RMS at the Department of Pediatric Surgery and the Division of Pediatric Oncology, University Hospital, Lund, Sweden. In 18 of these patients (6 girls and 12 boys, age range 1–17 years) chromosome analysis of tumor samples was attempted. Electron microscopy was routinely used on both cytologic and histologic specimens. The immunohistochemical analyses were performed with desmin, myoglobin, and during the last years also myo-d. The staging was according to IRS surgical histopathologic criteria [8]. The diagnosis was

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**TABLE I. Clinical and Cytogenetic Data on 18 Rhabdomyosarcomas**

| Case/sex/age[a] | Subtype[b] | Localization | Follow-up in years | Clinical course[c] | Sample[d] | Karyotype |
|---|---|---|---|---|---|---|
| 1/M/14 | Alv | hand | 2 | DD | TB P | 87–93,XXYY,+2,der(2)t(2;13)(q37;q14t)t(12;13)(q15;q22)x2,-3, der(8)t(8;14)(q13;q24)x2,+11,del(13)(q14;q22)x2,-14,-14, der(14)t(14;17)(q24;q21)x2,–15,der(16)t(8;16)(q13;p13)x2, del(19)(q13)x2,+1-3mar[11] |
| | | | | | BM M | 91,XXYY,der(2)t(2;13)t(12;13)x2,-3,-5,der(8)t(8;14)x2,+11, +11,del(13)x2,-14,-14,-15,der(16)t(8;16)x2,del(19)x2, +2mar[4]/46,XY[6] |
| 2/F/10 | Alv | thigh | 4 | AW | FNA P | 118,XXXXX,+X,+X,add(1)(p11)x2,+2,-3,-4,-4,+5,+5,-6,-7,+8, +9,-10,-11,+12,-14,-16,+20,+20t,+20,-21,+22,+der(?)t(?;5) (?;q15)x2[2] |
| | | | | | TB P | 118,idem[2]/46,XX[16] |
| 3/M/15 | Alv | neck | 1.5 | DD | BM P | 42–44,XY,-22[cp3]/46,XY[22] |
| 4/M/15 | Alv | neck | 1.5 | DD | TB P | 46,XY,t(4;17)(q11;p11),t(8;16)(q11;p13)[13]/46,XY [7] |
| | | | | | TB M | 70–73,XXY,+1,t(4;17)(q11;p11),+der(4)t(4;17),+5,+6,t(8;16), -10,-17,add(17)(p11),-19,inc [6]/46,XY[19] |
| 5/F/3 | Alv | thigh | 1.5 | AW | TB P | 46,XX[26] |
| 6/F/9 | Alv | leg | 1 | AW | FNA P | Failure |
| 7/M/2 | Alv | pelvis | 1 | AD | BM P | 88–93,XXYY,add(1)(p35)x2,+2,der(2)t(2;13)(q35;q14)x2, add(3)(q12),add(3)(q21),-8,-9,add(10)(q22),-12,-13,-13,-13, -13,-15,-19,inc[4] |
| 8/M/9 | Alv | head, neck | 0.5 | AD | TB P | 48,XY,+8,+?add(11)(p11),del(16)(q22)[7]/83-87,XXYY,+1,-3, -4,-5,-6,-7,-13,-14,-17,?i(17)(q10),-18,-18,-21,25-200dmin[5] |
| 9/M/4[e] | Emb | orbita | 12 | AW | TB M | 100,XXYY,+2,-6,+7,+8,+8,+8,-9,+11,+12,+12,+12,-13,+14,-15, -15,+18,+22,+22,+22[23]/46,XY[2] |
| 10/F/1 | Emb | retroperitoneal | 1 | DD | TB M | 46,XX,der(22)t(17;22)(q21;q13)[24] |
| 11/F/3[f] | Emb | epipharynx | 9.5 | AW | TB R | 46XX,t(1;11)(p13;q23),inv(5)(q22q33),inv(7)(p11q22),inv(12) (q21q24),inv(18)(p11q23)[2]/46,XX,t(2;4)(p15;q21),t(6;14) (q15;q21)[2]/46,XX [2] |
| 12/M/8 | Emb | orbita | 6.5 | AW | TB P | 54-55,X,+X,-Y,+2,+5,del(7)(q23),+8,+8,+10,+11,+11,+11,+13, der(16)t(11;16)(q13;q24),+20[6]/46,XY[3] |
| 13/M/9 | Emb | epipharynx | 4.5 | AW | TB P | 46,XY[25] |
| 14/F/8 | Emb | mandible | 4 | AD | FNA P | 46,XX,t(7;12)(q22;p13)[3]/46,XX[6] |
| 15/M/2 | Emb | neck | 2.5 | AW | FNA P | Failure |
| 16/M/2 | Emb | urinary bladder | 1.5 | AW | TB P | 57,XY,+der(1)t(1;11)(p10;q10),+2,+7,+8,+add(12)(q24),+13, +14,+17,+add(18)(q12),+20,+mar[6]/46,XY[4] |
| | | | | | TB P | 60,XY,-X,-1,-3,-4,-5,-6,-9,-10,+13,+15,-16,-21,-22[cp4]/46, XY[11] |
| 17/M/13 | Emb | thorax | 12 | AW | TB P | 44-46,XY,add(1)(p?),+del(1)(p11),-4,add(5)(p?),del(6)(q?), -10,-11,add(12)(q?),add(17)(p?),add(17)(q?),add(20)(p?), +1–5mar |
| 18/M/17 | Pleo | scrotum | 2 | DD | TB M | 89-92,XXYY,-2,-3,-5,-14,-15,+add(17)(p11),+22,+8–10mar, inc [4]/46,XY[2] |

[a]Age at diagnosis
[b]Alv = alveolar RMS, Emb = embryonal RMS, Pleo = pleomorphic RMS
[c]AW = alive without disease, AD = alive with disease, DD = dead of disease
[d]TB = tumor biopsy, BM = bone marrow, FNA = fine needle aspiration, P = primary tumor, R = local recurrence, M = metastasis
[e]Previously published by Olegård et al [16]
[f]Analyzed after radiotherapy

embryonal RMS in 9 cases, alveolar RMS in 8 and pleomorphic RMS in 1.

Cell cultures from tumor biopsies and fine needle aspiration (FNA) biopsies were processed for cytogenetic analysis as described by Mandahl et al [9] and Åkerman et al [10]. All cell cultures were harvested within 10 days. Bone marrow samples were cultured as cell suspensions for 48 hours before harvest. The chromosomes were G-banded with Wright stain, and karyotypic descriptions were according to ISCN (1995) [11].

## RESULTS

The cytogenetic, histopathologic, and clinical data are summarized in Table I. Clonal chromosome aberrations were found in 14 of the 18 tumors. An abnormal karyotype was found in 7 of 9 embryonal RMS, in 6 of 8 alveolar, and in the sole case of pleomorphic RMS. When the cytogenetic analysis was made on FNA samples the relative frequency of failures, i.e. when no karyotype could be produced, was higher (2 of 4) compared to the biopsy specimens (1 of 16).

## DISCUSSION

Prior to the present study, only 83 short-term cultured cytogenetically abnormal RMS have been reported [5]. Of these, 43 were classified as alveolar RMS, 21 as embryonal RMS, 3 as pleomorphic RMS and 1 as mixed alveolar/embryonal RMS. For the remaining 15 cases the subtype was not specified. A variety of chromosomal changes have been identified. The chromosome number has ranged from 30 (hyperhaploid) to 108 (hypopentaploid), with most of the cases displaying a near-diploid or near-tetraploid chromosome count. The most frequent and most characteristic karyotypic alteration is the t(2;13)(q35;q14) and variants thereof. This translocation, which so far has not been detected in any other type of solid neoplasm, is particularly associated with the alveolar subtype. Of the 43 previously reported alveolar RMS, 35 (81%) were found to display this abnormality. However, the same translocation has been reported in 2 of the 21 (10%) embryonal RMS, in the single case of mixed alveolar/embryonal RMS and in 3 of the 15 unclassified RMS. Albeit the subclassification of RMS may be difficult these findings indicate that the t(2;13) is not specifically associated with alveolar morphology [5].

In the present series, chromosome aberrations were found equally often in alveolar and embryonal RMS. The t(2;13) was found in 2 of 6 alveolar RMS with clonal aberrations, but not in any of the other RMS subtypes. Furthermore, one alveolar RMS (case 8) had a large number of double minute (dmin) chromosomes, a cytogenetic sign of gene amplification. In light of the recent demonstration that the critical fusion gene may be present on dmin chromosome [12], one could speculate that also our case had a cytogenetically undetectable amplified fusion gene. As no material was available for molecular analysis, this hypothesis could not be tested. Of the other 3 cytogenetically aberrant alveolar RMS one had monosomy 22 as the sole change (case 3), one had a hyperpentaploid karyotype with few structural aberrations (case 2) and one had a pseudodiploid karyotype with t(4;17)(q11;p11) and t(8;16)(q11;p13) as the only clonal rearrangements (case 4). Neither the monosomy 22 in case 3 nor the translocations in case 4 have been reported previously in RMS.

It has been suggested that there is a clear difference in the clinical features of alveolar RMS with and without the t(2;13) [13]. From a series of 14 alveolar RMS with t(2;13) Douglass and coworkers [13] concluded that t(2;13) was associated with a truncal location of the tumor, higher age (median 15 years) at diagnosis, higher frequency of occult primary tumors and more aggressive clinical course. Of the 2 cases of alveolar RMS with t(2;13) included in the present study, however, one was only 2 years old (case 7) at diagnosis, one tumor originated from the hand (case 1), and none of the patients presented with occult primary tumor. Obviously, more data are needed before any firm conclusions regarding the clinical significance of the t(2;13) can be drawn.

Three of the embryonal RMS (case 9, 12, 16) had hyperdiploid or hypertetraploid karyotypes with few structural rearrangements. Such karyotypes, when not containing the t(2;13), have previously only been described in embryonal RMS and appear to be strongly associated with this subtype. Interestingly, this cytogenetic pattern seems to be particularly frequent in pediatric neoplasms, e.g. acute lymphoblastic leukemia, infantile fibrosarcoma, Wilms' tumor, and ependymoma [5]. In acute lymphoblastic leukemia, hyperdiploidy is associated with good prognosis [3] and also in the present study this aberration pattern was associated with a less aggressive clinical course. Cases 12 and 16 are alive and without evidence of disease after treatment for their primary tumors, and case 9 remained disease-free for 12 years after treatment for a primary orbital embryonal RMS.

Three embryonal RMS exclusively displayed structural abnormalities. In case 10, an unbalanced translocation between chromosomes 17 and 22 was the sole aberration. Chromosome 22 rearrangements, leading to disruption of the *EWS* gene, have been described in RMS [14], but in our case no *EWS* alteration could be detected at molecular analysis (data not shown). In case 11, two unrelated clones were detected. As the sample was taken after radiotherapy we believe it is reasonable to assume that one or both of the clones were caused by the clastogenic therapy and hence were not representative of the tumor parenchyma. The t(7;12)(q22;p13) that was seen as the sole abnormality in case 14 has not been reported before in RMS. However, it could be noted that another embryonal RMS has an unbalanced t(3;7) with the same breakpoint on chromosome 7 as in our case [15].

The single case of pleomorphic RMS displayed a highly complex clone (case 18), which is in agreement with data on 3 previously published cases of this subtype. So far, no consistent chromosome aberration has been detected among pleomorphic RMS.

In conclusion, chromosome abnormalities are readily detected after short-term culture of tumor cells, preferrably obtained from surgical tumor biopsies, from all histopathologic subsets of RMS. The karyotypic pattern is already a valuable adjunct in the differential diagnosis between alveolar RMS and other subtypes. Furthermore, the still limited data indicate that the karyotypic pattern may be of prognostic significance.

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